Influence of green salting and pretanning operations on the protein constituents of cattle hide

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INFLUENCE OF GREEN SALTING AND PRETANNING OPERATIONS ON THE PROTEIN CONSTITUENTS OF CATTLE HIDE

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Different protein constituents have been determined in fresh cattle hide and in hides subjected to some of the beam house operations. Areawise variation in the protein constituents has been studied in five different locations on a side. Salt soluble proteins (10% NaCl) and lime soluble proteins (one-half saturated lime) are found to be present to the maximum extent in the neck region and to the minimum extent in the butt area. Salt soluble coagulable proteins are removed partially after green salting and subsequent washing of the hide and are almost completely removed after pretanning operations. About 35% of extractable lime soluble protein initially present in fresh hides is retained by pickled pelt; it may influence the properties of the finished chrome leathers. After pickling, the collagen content of the hide is raised by about 20%.

Determination of protein constituents in different pickled pelts obtained from hide samples treated in different ways indicates that removal of globular protein is comparatively less in air dried hide than in wet salted hide. In spite of different initial treatments, the protein constituents in the pelt samples vary only within a narrow range.

Besides fibrous protein, the basic leather making substance, small amounts of nonfibrous proteins or interfibrillary proteins are present in hides. The presence of nonfibrous proteins in hides and in pelts to different extent is considered to have significant influence on processing. Investigations on the interfibrillary proteins[1-4] have revealed that the globular proteins or serum proteins (extractable with 10% salt solution) and the mucopolysaccharides (mucoid material extractable with one-half saturated lime) are the most important components that considerably influence the rehydration of dry hides and other pretanning operations. But precise information regarding the presence of these interfibrillary proteins in pelt just before tanning and their influence on the quality of the finished leather is rather limited.

Data on nonfibrous proteins secured by Tancous[5] are illustrative but objections may be raised[6] about the procedure followed by her to estimate the globular proteins. Debeukelaer and Marbach[7]

followed a procedure in which the hide was first extracted with 10% NaCl to remove the globular proteins followed by extraction with half-saturated lime to eliminate the lime soluble proteins. The residual material was then treated with 0·1% HCl at 85°C for two hours. Keratin and elastin were removed as residue, collagen was estimated from the filtrate by direct Kjeldahl estimation. Thabaraj *et al*[8] followed a procedure where albumin, globulin, mucoid and collagen of a goat skin were estimated. In their study the value for collagen was obtained by converting the data on hydroxyproline.

In the present study, Debeukelaer and Marbach's method has been followed up to the extraction of lime soluble mucoid material and then collagen was estimated by the Neuman and Logan method.[9]

An attempt has been made to find out the extent of removal of interfibrillary proteins from the hide and the variation in protein constituents of the hide after pretanning operations.

Materials and methods

Freshly slaughtered hides obtained from aged cattle were collected from a slaughter house immediately after flaying.

Analysis for different protein components of hide

The hide piece was dehaired by first clipping the hair and then shaving it off. Excess flesh and fat was removed by careful fleshing. The hide piece was then chopped into small pieces and minced in a hand operated mincing machine in the presence of ice. About 10 g. minced material was used for extraction. The minced material was taken in a shaking bottle and extracted with 10% salt solution : : (hide: salt solution, 1:10) for one hour in a mechanical shaker. This was then centrifuged at 2000 r.p.m. for 15 minutes and the supernatant was decanted off through glass wool. Extraction was repeated twice with a fresh quantity of 10% salt solution using 1 part of Merthiolate per 10,000 parts of solution as an antiseptic during the extraction. The residue was washed twice with 10% salt solution and the filtrate and washings were combined and made up to a required volume. The filtrate contained globular proteins and non-protein nitrogen (A). An aliquot of the filtrate was analysed for nitrogen by Kjeldahl method. Trichloracetic acid was added to another aliquot of the filtrate to give a final concentration of 4% of the acid[15] and the coagulable proteins were coagulated by heating over a water bath. This was filtered. The filtrate was then analysed for nitrogen by the Kjeldahl method which gave the value for non-protein nitrogen (B). Globular protein was calculated from the difference of A and B.

The residue after the extraction of globular proteins was extracted with half-saturated lime (1:10) for one hour and centrifuged at 2000 r.p.m. for 15 minutes. The supernatant was decanted through glass wool. Two more extractions with fresh quantity of half-saturated lime were carried out. The residue was washed with half-saturated lime and the filtrate together with the washings was collected and made up to a known volume. An aliquot was taken for nitrogen estimation (Kjeldahl method)

and this gave the value for lime soluble protein nitrogen. Kjeldahl analysis was always done in duplicate. 4% boric acid and 0·01 *N* HCl were used to trap and titrate the ammonia released.

The residue was washed well with distilled water, dried in a basin to constant weight and powdered. A portion of it was digested with 6 *N* HCl for 18 hours at 105°C in a sealed tube and the hydrolysate was made up to a known volume (a separate moisture estimation was made). Percentage of collagen was calculated by estimating hydroxyproline by the Neuman and Logan method[9] using the conversion factor 7·52.[10] The hydroxyproline method is preferred as it gives a more direct measure of collagen than the Kjeldahl method. The remaining protein constituents have been termed as unextractable non-collagenous protein which may include keratin, elastin and unextractable interfibrillary proteins.

Experimental procedure and results

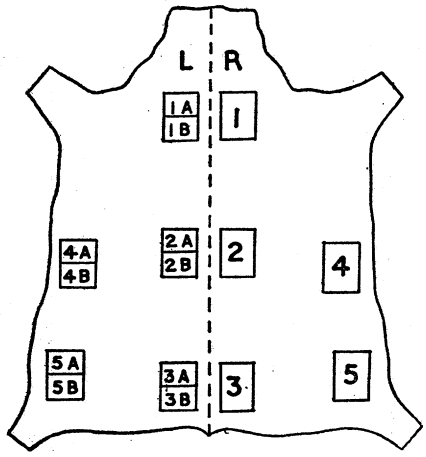
Locational variations of the interfibrillary proteins in the hide and the extent of their removal after pretanning processes

Freshly slaughtered hide was green fleshed and samples were cut from the right hand side in different locations (Fig. 1) and kept in polyethylene bags in a freezing box at 0°C till they were analysed. The left side was then cured by wet salting and stored for a period of 3 weeks. Samples were then cut from different sites of the salted side identical with those of the fresh samples (1A, 2A, 3A, 4A and 5A). The samples were washed in water to remove excess salt and then analysed.

The left side was then soaked in water for a period of six hours and limed for 40 hours in a liquor comprising 2·5% Na_2S, 0·5% ammonium sulphate, 8% lime and 350% water. The side was then unhaired, fleshed, washed well and delimed with 1% ammonium sulphate and 150% water for about one hour. The side was bated with 0·5% CLRI bate No. 2 for 30 minutes, scudded and then washed. Pickling was done with 1·25% H_2SO_4, 5% salt and 80% water. Samples were then cut from different positions (1B, 2B, 3B, 4B and 5B). These samples were kept at 0° for a few days till they were taken for analysis. Results obtained are given in Table 1.

Effect of different treatments of the hide on the extraction of interfibrillary proteins during pretanning processes

To find out the influence of the presence of different amounts of non-fibrous proteins on the properties of upper leather it is necessary to have pelts with varying



A—Salted and washed B—Pickled

FIG. 1. Sampling positions in different areas of the hide

Table 1

DIFFERENT PROTEIN CONSTITUENTS IN HIDE AFTER WET SALTING AND PRETANNING OPERATIONS*

Pre-tanning operations	Location	Salt soluble protein N	Non-protein N	Lime soluble N	Collagen N	Unextractable non-collagenous N
		% of total N				
Fresh hide	1	4·5	2·2	2·6	71·3	19·4
	2	3·4	2·2	1·9	72·6	19·9
	3	2·1	1·6	1·7	76·0	18·6
	4	2·8	2·1	2·4	72·0	20·7
	5	2·9	2·3	2·2	71·5	21·1
	Average	3·14	2·08	2·16	72·68	19·94
Salted & washed hide	1A	2·5	2·0	2·2	73·2	20·1
	2A	1·9	1·8	1·7	75·0	19·6
	3A	1·0	1·4	1·6	77·4	17·6
	4A	2·0	1·5	2·1	74·5	19·7
	5A	2·2	1·8	1·9	73·5	20·6
	Average	1·92	1·7	1·9	74·7	19·96
Pickled pelt	1B	0·06	1·2	0·84	86·8	11·10
	2B	0·07	1·1	0·87	87·8	10·16
	3B	0·04	0·89	0·63	89·0	9·44
	4B	0·04	0·87	0·71	87·4	10·98
	5B	0·05	1·10	0·79	86·20	11·86
	Average	0·052	1·03	0·77	87·44	10·71

* Data presented are the average values of samples from two hides.

amounts of these interfibrillary proteins. To obtain such pelts, hide pieces were treated in different ways as mentioned below:

1. *Raw hide*: Fresh raw hide sample (taken from position adjacent to location 3) was kept at 0°C in a polyethylene bag for about a week.

2. *Air dried*: Hide piece (taken from location adjacent to 1) was dried in the laboratory at room temperature (about 30°C).

3. *Dry salted*: Hide piece (taken from location adjacent to 2) was cured with 35% salt and then dried at room temperature.

4. *Extracted with 10% salt*: The hide piece (taken from location adjacent to 3) was extracted thrice with 10% salt solution and then cured with salt.

5. *Extracted with 10% salt and half saturated lime*: The hide piece (taken from position adjacent to 3) was first extracted thrice with 10% salt solution and

thrice again with half saturated lime solution and then cured with salt.

6, 7 & 8: These samples (taken from locations adjacent to 1 and 2) were applied with salt and stored in wet salted condition.

Processing

One fresh hide piece was soaked in water for a few hours. Liming, deliming, bating and pickling were done in a similar way as in the previous experiment. Dried hide was soaked for 3 days and the dry salted hide was soaked for two days in water in the presence of an antiseptic. The following procedures upto pickling were the same as above.

The sample extracted with salt solution prior to salting was soaked in water for about 4 hours and processed as above. The sample extracted with 10% salt and half saturated lime prior to curing was also processed as above. Samples 6, 7 and 8 were soaked in water for about 6 hours and then limed in different ways. Standard quality upper leathers could be produced by adopting any of the following liming processes.

Sample 6: *Short liming*: A soaked hide piece was taken in lime liquor of the following composition: 4% Na_2S, 6% $Ca(OH)_2$, 3% Hypo and 150% water. The piece was treated in this bath in drum for a period of 6 hours, the drum being run for 10 minutes every hour.

Sample 7: *Painting*: A soaked hide piece was painted on the flesh side with a paste of 2·5% Na_2S, 12% $Ca(OH)_2$ and 20 parts water and left overnight. The next morning the hide was unhaired and then put in a liquor containing 1·5% Na_2S and 300% water with occasional handling. Next day, the hide piece was taken out, fleshed, washed and then delimed.

Sample 8: *Pit liming*: A soaked hide piece was limed in a liquor comprising 1·5% Na_2S, 5% $Ca(OH)_2$ and 300% water for 72 hours with occasional handling each day.

After the usual deliming, bating and pickling, all the samples were analysed for their protein constituents and the results obtained are presented in Table 2.

Table 2

DIFFERENT PROTEIN CONSTITUENTS IN PICKLED PELTS OBTAINED FROM HIDES TREATED IN DIFFERENT WAYS

Treatment	Salt soluble protein N	Non-protein N	Lime soluble protein N	Collagen N	Unextractable non-collagenous N
	% of total N				
Raw hide	0·06	1·56	0·95	85·10	12·33
Air dried	0·20	2·14	0·85	82·40	14·41
Dry salted	0·10	1·26	0·71	84·00	13·93
Salt extracted	0·04	1·21	0·74	86·80	11·21
Salt+lime extracted	0·04	1·30	0·64	86·10	11·92
Short liming	0·04	1·45	0·90	85·80	11·81
Normal liming	0·06	0·96	0·80	86·50	11·68
Long liming	0·05	1·25	0·59	88·00	10·11

Discussion

Table 1 shows that the protein constituents in different locations of a fresh raw hide may differ to a certain extent. The amount of globular (salt soluble) protein nitrogen is found to be maximum in the neck area (position 1) and is gradually reduced towards the butt area (position 3) where it is minimum. The amount of globular proteins in the belly (position 4) and shank (position 5) areas is in between the values for the neck and butt areas. Variation in lime soluble protein in different areas of the hide is however small, but the maximum amount of lime soluble protein nitrogen also is found in the neck area and the minimum in the butt area. Tancous noted a reduction is globular proteins from neck to butt area in calf skin[5] and steer hide.[11] Globular protein nitrogen and lime soluble nitrogen were found to vary with location and age of skins.[12]

According to Rosenthal,[13] calf skin yielded about 4·5% coagulable nitrogen and 2·8% mucoid. Debeukelaer and Marbach[7] reported 2·8% salt soluble globular proteins and 1·0% lime soluble protein in fresh steer hide. Following a similar extraction method, 4·9% globular proteins and 5·7% mucoid (on dry goat skin) were found in fresh goat skin.[8] In the present study, the average values for salt soluble globular proteins and lime soluble proteins are 3·14% and 2·16% respectively. It is however recognised that calf and goat skins may have more interfibrillary proteins than aged cattle hide.

Non-protein nitrogen does not vary much with location on the hide except that it is minimum in the butt area. Collagen nitrogen, on the other hand, is found to be maximum in the butt area and minimum in the neck area. Tancous reported 73·1% collagen nitrogen (by Kjeldahl estimation) in calf skin[5] and 86·3% collagen nitrogen in steer hide.[11] According to Debeukelaer and Marbach[7] collagen nitrogen in steer hide was 89·6%. Following the hydroxyproline method, 77·5% collagen (on dry goat skin) was found by Thabaraj *et al*[8] in goat skin. In the present investigation, the average collagen content of cattle hide appears to be 72·7%. Unextractable non-collagenous protein nitrogen in fresh hide is found to vary slightly within the range 18·5-21·0%.

After green salting and washing of the hide, a considerable proportion of globular proteins is found to be extracted and removed. Non-protein nitrogen and lime soluble proteins are also removed to some extent. The amount of collagen nitrogen, however, appears to be slightly increased, but this may be a consequence of the removal of non-collagen nitrogen and the fact that the removal is expressed as percentage of the total nitrogen. Removal of globular proteins and lime soluble proteins to different extents[5,8,11] and the slight increase in collagen content[5,7,11] after salt curing and soaking having been reported by other investigators. Okamura and Kawamura,[14] however, observed no essential difference in collagen content between green and salted hides. It may further be noted that unextractable non-collagenous protein nitrogen remains unaffected by wet salting and washing (Table 1). The trend in areawise variation of different protein constituents of the hide after salting and washing is somewhat similar to that of fresh hide.

After pretanning operations i.e., after pickling, globular proteins are found to be mostly removed (98%) from the hide. Lime soluble proteins and non-protein nitrogen are found to be removed after pickling to the extent of 64 and 50% respectively. Collagen nitrogen, on the other hand, is found to be increased by about 20% on the collagen content present in fresh hide. But the value for collagen nitrogen appears to be slightly low. Thabaraj *et al*[8] reported the removal of about 80% globular proteins and 59% lime soluble protein from goat skin after pretanning operations. According to Tancous[5], 89% globular proteins (including mucoids) are removed from calf skin after pickling. An increase in collagen nitrogen by 29% was noted by her. The amount of unextractable non-collagenous protein is found to be appreciably reduced (46%) in pickled pelt. Although it is quite possible to have certain differences regarding the extent of removal of different interfibrillary proteins depending on the type of hide or skin, their initial protein constituents, processing operations and the existing temperature, it is apparent from the present data that the extractable globular proteins are removed to a great extent after pretanning operations but about 35% lime soluble proteins remain in the pelt that is ready for tanning.

In order to produce pelts having varying amounts of interfibrillary proteins, hide samples were treated in different ways so that some of them e.g. raw, air dried and dry salted samples might retain more and others might have less amount of interfibrillary proteins than a normal wet salted hide. After soaking, the samples were put through the same pretanning operations. The wet salted samples that were limed in different ways were also delimed, bated and pickled along with other samples. Data presented in Table 2 indicate that irrespective of the removal of interfibrillary proteins to varying extents in precuring stage, practically the same small amount of globular proteins is present in all samples except in the air dried and dry salted ones where the values are slightly higher. It is possible that the interfibrillary proteins present in these samples got partially denatured due to drying thus affecting the removal of the salt soluble proteins. Lime soluble proteins present in the pelts vary within a small range although it appears that the removal is slightly more in the case of the samples initially extracted with 10% salt solution and half saturated lime and also in the other case, where the sample was subjected to long liming. Collagen nitrogen appears to be slightly less in air dried sample but slightly more in the case of sample subjected to long liming. On the other hand, unextractable non-collagenous protein nitrogen is found to be slightly higher in air dried sample and slightly lower in case of the sample subjected to long liming.

The results of the present investigation thus point out that wet salted hide, after normal pretanning operations, retains small amounts of extractable globular proteins which may be considered to have little effect on the properties of finished leathers. A considerable proportion of lime soluble protein is, however, retained by the hide after pretanning processes which may influence leather quality but this requires further confirmation. But it is felt that any

drastic treatment to remove further amounts of lime soluble proteins may probably act also on collagen in some way thus impairing leather quality irrespective of the quantity of the non-fibrous proteins left in the pelt. The effect of the presence of unextractable non-collagenous proteins has not been considered in the present study.

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